12 Data Analysis Issues

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CONTENTS

This chapter discusses recommendations from the Workshop participants on existing methods and approaches for statistically assessing exposure and effects to pollinators using both laboratory and field tests. In a few cases, broad suggestions are discussed on how to examine, present, and evaluate data from these tests. Participants identified a need for additional statistical analysis tools for evaluating data from existing study designs and results to aid in the design and conduct of future study protocols. However, neither the discussions of the Workshop nor established guidance documents (e.g., EU's Dir 91/414 and EPA Part 158 Test Guidelines) provide suggestions or case study illustrations detailing appropriate approaches for statistically examining data from both short- and long-term laboratory and field tests. An exploration of analytical methods most appropriate for evaluating data would serve to inform regulatory authorities, agrochemical registrants, and researchers engaged in such studies. This chapter provides a brief overview of the types of statistical issues relevant to evaluating the potential effects of pesticides on pollinators that will be addressed by attendees of the Workshop through a subsequent effort, at a later date (note: details will be provided in a separate document through SETAC publications). The intent is to highlight issues of interest to risk assessors during the data analysis and risk characterization phases specifically with data generated on bees for use in an ecological risk assessment.

12.1 STUDY DURATION

Decisions regarding study duration and dosing time will impact any statistical model applied to the data, including dose–response models, and can have a large impact on the statistical inferences drawn from the data. The duration of some of the proposed laboratory-based chronic studies is 10 days. However, the implications of both longer and shorter durations have not been tested either in terms of their ability to detect subacute/chronic effects or the relevancy of laboratory-based studies to field-based studies of longer duration.

12.2 REPLICATES AND DOSING

Questions around the number of bees per replicate, the number of replicates per dose, is an element in both laboratory and field studies that requires consideration. In laboratory-based studies, key issues include the clear definition of treatment units, estimation and interpretation of between-treatment variance, and temporal variation over the course of the test. In semi-field and field studies, the concept of a replicate and whether

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information from multiple hives on the same field can be considered true replication versus pseudoreplication is critical to the calculation of variation in these tests.

Dosing in laboratory-based studies is more standardized than in field studies. Dose levels in a laboratory-based studies are carefully selected to cover the range of possible effects to allow the estimation of a dose–response function. Whereas individual bees may be "dosed" in laboratory-based study, in field studies there can be uncertainty regarding the extent to which bees are actually exposed to test material. Examination of raw data from tests of such a design can result in a visual non-monotonic dose–response relationship. Methods for interpreting this information, and the implications for selection of dose levels, are of interest to the development of subsequently applied statistical models.

12.3 LONG-TERM TESTS

In chronic tests (10/14-day test, semi-field, and field), the test is generally designed to be sensitive to sublethal effects, and consequently treatment levels and duration may be different from lethality tests. The length of the test and its influence on the calculation of statistical endpoints and uncertainty in the model-based endpoints should be examined. However, high variability in measurement endpoints and low replication can confound efforts to detect statistically significant effects. Field studies have the advantage of extending for longer periods than other tests, but the length of these tests should be examined with respect to bee life stage and the extent of an effect that would be necessary to impair the colony as a whole. Consideration may need to be given to cumulative dosing effects in longer-term studies. In addition, how issues of temporal variation, temporal correlation, and trends are assessed for multiple endpoints are areas which should be more standardized to ensure greater consistency and comparability between studies.

12.4 STATISTICAL MODELS

Many methods are available for dealing with dose–response information. Selection of the model structure is important and mathematical approaches for treating study data and resulting curves are issues. Classic probit and logit models are typically chosen, but given biological and experimental variations, the choice of model or experimental design can result in differing LC50 and EC50 estimates. Methods and approaches for dealing with differing results will be addressed in the anticipated analysis.

In brood tests, mortality is expressed as a percentage of the reference population after an adjustment according to the Abbott formula. However, other statistical methods and variance calculations are available, although no sensitivity studies on the test results have been conducted to date to determine the appropriateness of the models used to fit the data. Statistical methods for estimating the probability of survival at a specific age may be appropriate for these data, depending on the experimental design established for the test. In semi-field and field tests, which are typically hypothesis-based as opposed to regression-based study designs, questions include whether there are appropriate time-series models for testing for long-term trends in multiple endpoints, and how non-linear or episodic time-series data are treated. Use of specific statistical models may be more appropriate to evaluate long-term survival and hazard. Examination of survival functions for semi-field tests is an area of future research.

Through the review of several existing datasets, additional areas of analysis may be addressed, including treatment of controls or baseline effects. The anticipated work will examine approaches and interpretation of uncertainty in examining endpoints and output from tests. In addition to examining variability, an evaluation of uncertainty will include examples and case studies for interpreting results in light of the uncertainty estimates.